Investigation of Factors of Probable Significance in the Pathogenesis of Pneumonic Pasteurellosis in Cattle

R. G. Thomson, S. Chander, M. Savan and M. L. Fox*

ABSTRACT

Six groups of ten beef calves six to eight months of age were shipped from western Canada and observed untreated for one week after arrival. The following parameters were measured daily: body temperature, plasma fibrinogen, nasal bacterial mean colony counts of Pasteurella hemolytica and Pasteurella multocida, total and differential leukcoyte counts, packed cell volumes and the following, twice during the week: serum and nasal antibody titres to P. hemolytica and parainfluenza-3 virus. The lungs from 44 of the calves were obtained at post mortem and given a numerical score based on the degree of pneumonia present.

Animals were designated SICK and WELL according to body temperature and plasma fibrinogen. The SICK animals had higher nasal mean colony counts of P. hemolytica than the WELL animals. The SICK animals had lower levels of serum antibody to P. hemolytica than the WELL on day 1 but had a greater rise in titre over the week than did the WELL animals. Both groups were similar with regard to serum antibody to parainfluenza-3 virus and there was little change in these titres. The SICK animals had a much greater degree of pneumonia than the WELL.

The values of some of the parameters were combined with the data of previously studied animals in order to provide a comparison of SICK and WELL with larger numbers of animals.

RÉSUMÉ

Cette expérience visait à observer, sans les traiter, durant une semaine après leur arrivée de l'Ouest canadien, six groupes de dix bouvillons âgés de six à huit mois. On mesura quotidiennement les paramètres suivants: température, fibrinogène plasmatique, nombre moyen de colonies nasales de Pasteurella hemolytica et de Pasteurella multocida, comptages totaux et différentiels des leucocytes et hématocrite. Deux fois au cours de cette semaine, on procéda à la recherche du taux d'anticorps sériques et nasaux à l'endroit de P. hemolytica et du virus para-influenza 3. Lors de la nécropsie, on recueillit les poumons de 44 bouvillons et on les classa numériquement d'après le degré de pneumonie observé.

On classifia les bouvillons en MALADES ou SAINS, en se basant sur leur température et la teneur en fibrinogène de leur plasma. Les MALADES présentaient un nombre moyen de colonies nasales de P. hemolytica supérieur à celui des SAINS. La teneur du sérum des MALADES en anticorps à l'endroit de P. hemolytica s'avéra inférieure à celle du sérum des SAINS, le premier jour; elle augmenta cependant plus que celle du sérum des SAINS, au cours de la semaine. La teneur du sérum en anticorps à l'endroit du virus para-influenza 3 s'avéra semblable chez les sujets des deux groupes et ne manifesta pas de changement appréciable. Le degré de pneumonie s'avéra beaucoup plus marqué chez les MALADES que chez les SAINS.

*Department of Pathology (Thomson, Chander and Fox) and Department of Veterinary Microbiology and Immunology (Savan), Ontario Veterinary College, University of Guelph, Guelph, Ontario.

The work was supported by the Ontario Ministry of Agriculture and Food and by Agriculture Canada.

Submitted August 21, 1974.

On compara les valeurs de certains des paramètres à celles obtenues lors d'expériences antérieures, afin de pouvoir comparer les MALADES et les SAINS à un plus grand nombre de sujets.

INTRODUCTION

The relationship of stress, bacteria and viruses as etiological factors in the pathogenesis of "shipping fever" in cattle is not clear. It has become necessary to examine the interactions among the possible etiological factors in the early stage of the natural disease to determine which are of greatest importance in the initiation of the disease so that preventive or control measures can be developed.

In a previous study (26) on microbiological factors which might be related to the occurrence of pneumonia, animals shipped from western Canada were classified SICK (S) or WELL (W) according to body temperature and plasma fibrinogen values but in most, the presence or absence of pneumonia was not confirmed by post mortem examination. Also the data collected over a four week period was reanalyzed and it was shown that similar conclusions could be drawn from the data collected in the first week alone (error approximately 20%).

Therefore the present study was undertaken to relate factors which might be related to the occurrence of pneumonia to the degree of pneumonia present in the animals following examination of the lungs at the end of the observation period. The division between S and W animals was redefined, total and differential leukocyte counts and packed cell volume (PCV) were determined. In addition the following parameters were measured: body temperature, plasma fibrinogen, nasal mean colony counts (MCC) of *Pasteurella hemolytica* and *Pasteurella multocida*, serum and nasal antibody to *P. hemolytica* and parainfluenza-3 (PI-3) virus.

By discriminant analysis, an effort was made to predict S and W by using either day 1 or day 7 parameters only.

For as many parameters as possible, information was pooled from animals in the present investigation with that from the previous investigation including those referred to in the addendum (26), plus one other group. Thus overall comparisons between S and W animals could be made on larger numbers of animals than that reported previously.

MATERIALS AND METHODS

ANIMALS

Groups of beef calves six to ten months of age were purchased after they arrived by train from western Canada. Groups A to E (Table I) were described previously (26). Groups F and G were referred to previously in the addendum (26) and were purchased from the range, sampled in Saskatoon and then shipped to Guelph in order to determine the status of the parameters being measured prior to shipment from western Canada and to compare the findings after arrival in Guelph with animals in Groups A to E. Group H was handled in a similar manner to Groups A to E. Groups J to O were purchased as A to E but were housed each in two groups of five each in a stable in box stalls and were not outside after arrival as were Groups A to H (the letter I was not used to avoid confusion with numerals). The purpose of Groups J to O was to make similar observations as were made on A to H but to slaughter the calves after one week of observations, recover the lungs, determine the extent of pneumonia in each calf and relate the extent of pneumonia to the parameters measured.

CLASSIFICATION

Groups A to H were originally classified S and W on the basis of having a body temperature of at least 104.5°F for at least one day, followed within 48 hours by a plasma fibrinogen level of at least 800 mg % (26) for three successive days. To determine if the values for these criteria could be refined and related to pneumonia the computer selected the animals into S and W by: (a) classification of Groups J to O according to the above criteria and (b) combinations of temperature levels (105°F, 104.5°F, 104°F) and fibrinogen levels (600, 700, 800 mgm %). Thus the specific animals in each classification could be identified and their lung scores (see below) could also be identified. The best fit of temperature, fibrinogen and lung scores occurred at a temperature level of 104°F and fibrinogen level of

TABLE I. Groups of Animals and Classification

Animals				Classification			
				Original		Final	
Groups	Date	Total	Used for Lung Scores	SICK	WELL	SICK	WELL
A	Oct/67	10		5	5	6	4
B	Nov/67	10		7	3	7	3
C	Jan/68	10		9	1	9	1
D	Feb/68	10		1	9	2	8
E	Mar/68	15		11	4	12	3
F	Nov/68	10		5	5	6	4
G	Jan/69	8[a]		0	8	0	8
H	Feb/71	10		3	7	3	7
J	Oct/71	10	5[b]			5	5
K	Nov/71	10	10			4	6
L	Dec/71	10	9[c]			9	1
M	Jan/72	10	0[b]			1	9
N	Feb/72	10	10			2	8
O	Mar/72	10	10			4	6
		143	44			70	73

Number used in overall comparison of S vs W[d] 68 + 71 = 139
Number [b] used to relate S vs W to lung scores 23 + 21 = 44

[a]Two animals removed from data because fibrinogen levels not recorded
[b]Fifteen animals were sold. therefore lungs not available
[c]One died on first day
[d]Four animals died on first two or three days and therefore insufficient data on all parameters

700 mgm %. The specific time relationship for temperature and fibrinogen was altered so that the three days of high plasma fibrinogen levels occurred within 72 hours of the temperature level of 104°F and not necessarily after the temperature rise.

After the analysis the daily records of all animals in Group A to H were reexamined in order to determine if the new criteria would change the classification reported previously. The previous report on Groups A to H did not deviate from the temperature of 104.5°F and three successive days of fibrinogen levels at 800 mgm % (Table I — original classification). As indicated in Table I four animals originally classified as W moved into the final classification of S in Groups A to H (note the difference in "original" and final classification). All comparisons of S and W animals in this paper (note "final" classification in Table I) are based on the criteria of S animals having a temperature level of at least 104°F and three days of plasma fibrinogen levels of 700 mgm % and with very few exceptions the required fibrinogen levels occurred on three successive days and during days 1 to 7.

Body Temperature

Body temperatures were recorded daily. Daily plasma fibrinogen levels were determined by the Biuret method (12) in Groups A to H and by the Fibrometer method (17, 25) in Groups J to O. The following statement compares the two methods (24):

"1. At low bovine plasma fibrinogen values i.e. 200 mg/100 ml, the Fibrometer method tends to overestimate the plasma fibrinogen.
2. At high bovine plasma fibrinogen values i.e. over 1,000 mg/100 ml, the Fibrometer method tends to underestimate the plasma fibrinogen.
3. In the normal bovine plasma fibrinogen range of about 400-800 mg/100 ml the Fibrometer method will give an estimate of the plasma fibrinogen very similar to that of the Biuret method.

A Biuret value of 1,000 (a significantly elevated plasma fibrinogen) will be in the range of 963-993 on the average by the Fibrometer method."

Nasal Mean Colony Counts

The MCC of *P. hemolytica* and *P. multocida* in the nasal flora were determined as reported previously (26) except that in the calculation of the MCC the value for "a" of 0-2 was used. Therefore all the MCC for Groups A to H were recalculated in order to have comparable results and were calculated as a weekly value recorded once as a day 7 value.

Serology

Serum samples and nasal secretions were collected on day 1 and day 7. Serum and nasal antibody levels to *P. hemolytica* and PI-3 virus respectively were carried out as described previously (20, 26).

Hematology

Packed cell volumes (PCV) were determined by the microhematocrit method. Total leukocyte counts were determined on a Coulter FN counter and differentials determined routinely.

Lung Scores

All calves were killed by day 12 of the study. The lungs were examined grossly and photographed. All discoloured areas were recorded on a stencil outline of the bovine lungs. The extent of lesions in each lobe of each lung was recorded on a numerical scale ranging from zero to five with zero being no lesions and five indicating involvement of the entire lobe. Each lobe could therefore receive a numerical score on the extent of involvement with lesions. The left lung with three lobes and the right with four would have a total of seven individual scores which could be added to give a total for each calf. The maximum value was therefore 35 and the minimum zero.

Some parameters were examined on the basis of the total lung score of each animal and not the S and W classification. An arbitrary division of the animals of GROUP I (see below) into three subgroups (*a, b c*) was made with subgroup *a* including scores of zero to ten (25 animals), *b* including scores of 11-20 (ten animals) and *c* including scores of 21-30 (nine animals).

Analyses

The relationship of the extent of pneumonia to the parameters measured was of particular interest and therefore Groups J to O were examined as one large group. The data from Groups J to O (except for lung scores, PCV and leukocyte counts) were then pooled with Groups A to H in order to increase the number of observations for each parameter common to all animals. For purposes of presentation, Groups J to O (those from which lungs were examined) will be designated as GROUP I and A to O (all groups) as GROUP II. The results in GROUP I will be presented first.

Temperature, fibrinogen, PCV, total and differential leukocyte counts were recorded and analyzed using actual values (the differential leukocyte counts were converted to absolute counts). For the MCC and serological data the data was transformed as follows:

$$X_1 = \text{MCC } \frac{P.\ hemolytica}{100}$$

$$X_2 = \text{MCC } \frac{P.\ multocida}{100}$$

X_3 = log [(reciprocal of dilution of serum antibody to *P. hemolytical*) + 1.0]
X_4 = log [(reciprocal of dilution of nasal antibody to *P. hemolytica*) + 1.0]
X_5 = log [(reciprocal of dilution of serum antibody to PI-3 virus) + 1.0]
X_6 = log [(reciprocal of dilution of nasal antibody to PI-3 virus) + 1.0]

The results recorded in tables in this paper are shown as the actual means and standard deviations but the t and significance values for parameters X_1 to X_6 are the values from analysis of the transformed data.

Differences in mean values for each parameter compared between S and W animals were tested by the t-test (21). Homogeneity of variances is required for the t-test and therefore an F-test for differences between variances of S and W animals for the same parameters used in the t-test was carried out. If the variance was significant ($P \leq .05$) thus invalidating the t-test the Welch T Prime approximation of variables (29) was used to determine significant differences between the mean values. In general the same results were obtained with the t-test and the T Prime test and in most instances the significance values recorded are from the t-test but if the Welch T Prime approximation is used to determine significance the significance values from this test are used and are designated as such in the tables.

As mentioned in the introduction it seemed useful to reduce the number of observations required to determine S and W in the previous report (26) and thus reduce the time and cost of processing samples. The data from GROUP II were analyzed for this purpose. About half the animals classified as S and W were selected and using their day 1 values for parameters

TABLE II. Comparison of Mean Bacterial Colony Count in the Nasal Flora Between SICK and WELL Animals of GROUP I

	Classification						
	SICK		WELL		Significance		
Organism	MCC[a]	SD[b]	MCC	SD	DF[c]	t[d]	P[e]
P. hemolytica	566	623	172	399	42	2.47	.01[f]
P. multocida	124	292	148	314	42	0.25	0.80

[a]Mean colony count
[b]Standard deviation
[c]Degrees of freedom
[d]t-test value
[e]Actual significance value
[f]Significance value by Welch T Prime test

X_1 to X_6 they were reclassified using discriminant analysis and the percentage error determined. The same was carried out with the day 7 values (the same MCC values were used on both days for this calculation). Then the X_1 to X_6 parameters of day 1 and day 7 respectively of the other half of GROUP II were used to classify that half of the animals as S or W and the percentage error determined. Various combinations of the parameters were used in an effort to discard some parameters in order to improve the accuracy.

Dead Animals

The data on the 13 calves which died or were killed *in extremis* during the observation periods from GROUP II were brought together and examined to look for factors common to these animals and to compare them as a group with the S and W animals. One died on day 1, two on day 2, one on day 4, three on day 5, one on day 6, two on day 7, two on day 8 and one on day 9.

RESULTS

Group I (Groups J to O in Table I)

Temperature — The mean daily body temperatures (Fig. 1) were significantly different ($P \leqslant .01$) between S and W on all days except days 5, 6 and 7 and the overall means were significantly different.

Fibrinogen — The mean daily plasma fibrinogen levels (Fig. 2) were significantly different ($P \leqslant .01$) between S and W on all days as was the overall mean.

Mean Colony Counts — The nasal bacterial MCC of *P. hemolytica* was significantly higher in the S than the W animals but the same was not true for *P. multocida* (Table II).

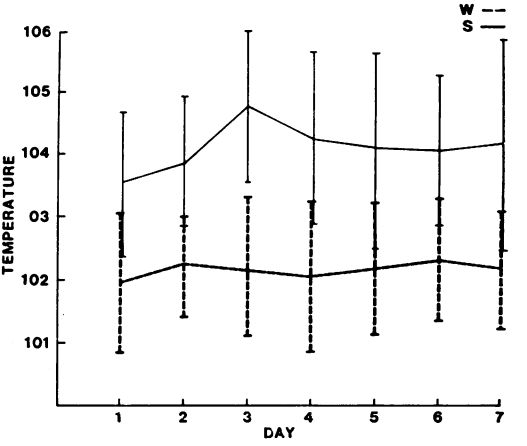

Fig. 1. Daily mean body temperature with standard deviations for SICK and WELL animals in GROUP I.

Antibody Titres — Comparisons of antibody titres were made between S and W animals on day 1 and day 7 in order to determine differences at these times and also to determine relative changes between day 1 and day 7. The serum IHA antibody levels to *P. hemolytica* was significantly higher ($P \leqslant .05$) in W than S animals at day 1 but not at day 7 (Table III). Differences in nasal titres to *P. hemolytica* were not significant. Neither serum nor nasal hemagglutination inhibition (HI) antibody titres to PI-3 virus were significantly different between S and W on day 1 or day 7 (Table III). The only significant change in serum and nasal antibody titres to *P. hemolytica* and PI-3 virus ($P \leqslant .05$) which took place *between* day 1 and day 7 was the serum antibody level to *P. hemolytica* in the S animals.

TABLE III. Comparison of Mean Antibody Titres Between SICK and WELL Animals of GROUP I on Day 1 and on Day 7

Agent		Day	Titre				Significance		
			SICK		WELL		DF[e]	t[d]	P[e]
P. hemolytica	— serum..	1	40[a]	35[b]	72	105	42	2.13	.04
		7	130	92	97	118	42	1.70	.10[f]
	— nasal	1	0	2	0.1	2	42	1.83	.07
		7	4	2	1	2	42	1.20	.23
PI-3 virus	— serum..	1	31	57	30	37	42	1.12	.27
		7	35	38	35	29	42	1.30	.18[f]
	— nasal...	1	18	29	10	10	42	.26	.80
		7	11	12	12	15	42	.55	.58

[a]Reciprocal of dilution
[b]Standard deviation
[e]Degrees of freedom
[d]t-test value
[e]Actual significance value
[f]Significance value by Welch T Prime test

Hematology — There was a significant difference in the PCV between S and W animals only on day 2 (Table IV). No significant difference was observed in the mean total leukocyte counts between S and W animals (Table IV) and only a few of the differential white cell counts were significant (Table V). The only definite trend observed was that the eosinophil counts in the S animals were significantly lower than in the W animals on each day (Table V).

Lung Scores of S and W Animals — The individual S and W animals are listed in Table VI with their lung scores and the mean scores for S and W. The overall mean lung score for all animals was 10.2 with a standard deviation of ±9.5, for the S animal the mean score was 17.6 and for the well 5.7. For 42 degrees of freedom the t value for the t-test was 8.80 and the actual significance was .00 with the S animals having the significantly higher scores. A test for equality of variances between the groups indicated no significant differences and therefore the t-test could be used for this data (21). These results clearly demonstrate the difference in the degree of pneumonia between S and W animals.

Parameters Based on Magnitude of Lung Score — Although the subgroups *a*, *b* and *c* were uneven in numbers of animals some comparison of parameters based only on lung scores was considered useful in the hope that characteristics of the animals with the greatest degree of pneumonia might be more closely identified and compared to those with the least degree of

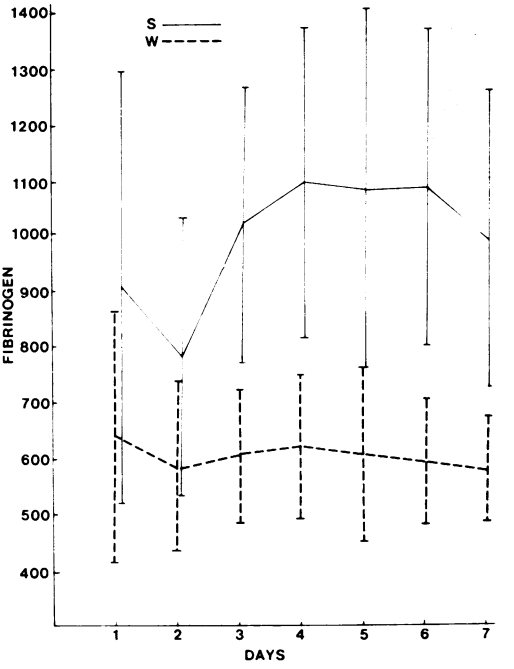

Fig. 2. Daily mean plasma fibrinogen levels with standard deviations for SICK and WELL animals in GROUP I.

TABLE IV. Mean Packed Cell Volumes and Total Leukocyte Counts of SICK and WELL Animals in GROUP I

	Packed Cell Volume						Total WBC			
	SICK			WELL			SICK		WELL	
Day	Mean	SD[a]	Range	Mean	SD	Range	Mean	SD	Mean	SD
1	39	4	30 — 47	41	4	33 — 49	9105	3772	9491	3542
2	35	3	28 — 42	38[b]	4	30 — 44	8295	2012	8952	2329
3	36	5	30 — 47	37	5	20 — 44	8414	1829	8904	3167
4	37	4	29 — 47	35	5	25 — 46	8814	1941	8600	2687
5	35	4	30 — 44	35	4	23 — 44	8814	1941	8600	2687
6	35	3	31 — 40	33	4	23 — 40	9376	2256	9000	2234
7	34	5	30 — 41	34	4	27 — 42	8886	2602	8927	3716
Overall Mean	36	3		35	3		8860	1600	8650	2200

[a]Standard deviation
[b]Significant difference ($P \leq .05$)

pneumonia. The temperature and fibrinogen levels of subgroups *b* and *c* are more closely associated with each other than with subgroup *a* (Fig. 3, 4). The total leukocyte counts do not differ greatly in the three subgroups (Table VII) and if graphed the lines overlap each other. Subgroup *c* has the highest number of band cells on days 3 and 4 and segmented cells on day 7. If graphed the lymphocyte and monocyte lines overlap each other. Subgroups *b* and *c* have much lower eosinophil counts than subgroup *a*.

The MCC of *P. hemolytica* is higher in subgroups *b* and *c* than in *a* and there is little difference between subgroups for *P. multocida*. The increase in serum antibody titre to *P. hemolytica* between day 1 and day 7 is greatest in group *c* (Table VIII). Also the mean titre to *P. hemolytica* on day 1 is lowest in *c*. There is little change in antibody titres to PI-3 virus between the subgroups (Table VIII). In general these differences between subgroups *b* and *c* on the one hand and *a* on the other are similar to the differences between S and W animals but are more indicative of the degrees of pneumonia than is S and W alone.

GROUP II (GROUPS A TO O IN TABLE I)

Temperature — There are significant differences between temperature values (Fig. 5) of S and W animals only on days 1, 3 and 4 as well as overall.

Fibrinogen — There are significant differences in plasma fibrinogen values (Fig. 6) between S and W animals on all days.

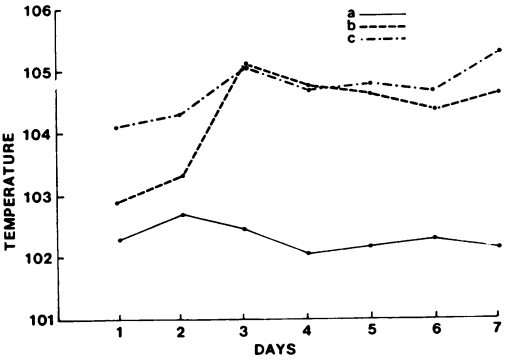

Fig. 3. Daily mean body temperature for subgroups a, b and c, divided according to lung scores, from GROUP I (a includes lung scores of 0-10, b, 11-20 and c, 21-30).

Mean Colony Counts — The MCC of *P. hemolytica* of the S animals is significantly higher than the MCC of the W animals whereas there is no significant difference with *P. multocida* (Table IX).

Antibody Titres — The serum antibody titre to *P. hemolytica* is significantly lower in the S than the W animals at day 1 but not at day 7 (Table X). The serum antibody titre to PI-3 virus is significantly higher at day 7 but not at day 1 in the W animals (Table X). In comparing serum and nasal antibody to *P. hemolytica* and PI-3 virus between day 1 and day 7 the significant changes ($P \leq .05$) were, (i) the serum antibody titre to *P. hemolytica* was higher on day 7 than day 1 for the S animals and for the W animals and (ii) the

TABLE V. Mean Differential Absolute Leukocyte Counts in SICK and WELL Animals of Group I

	Band				Segmented				Lymphocyte				Monocyte				Eosinophil			
	SICK		WELL		SICK		WELL		SICK		WELL		SICK		WELL		SICK		WELL	
Day	Mean	SD[a]	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
1	61	100	37	101	*3597*[b]	2644	1904	1620	*5622*	1972	6717	2522	70	150	136	164	*129*	135	294	238
2	*64*	91	12	56	*2599*	1173	1749	1203	6006	1631	6042	1460	119	97	111	125	*142*	160	349	288
3	71	189	29	62	2351	1511	1776	1034	6267	2120	6164	1306	102	116	109	91	*105*	177	320	262
4	65	210	7	34	2318	1142	1746	1222	6071	1935	6677	1282	82	130	83	115	*48*	65	257	221
5	52	93	10	48	2163	940	1783	996	6340	1869	6886	1812	*59*	80	142	132	*101*	115	331	321
6	97	200	38	119	2487	1307	2243	1931	6297	2126	6595	1306	*41*	52	130	110	*67*	75	330	291
7	111	229	35	139	2863	2601	2074	2015	5805	1689	6293	1462	70	93	76	75	*70*	69	358	427
Overall Mean	72	115	24	67	2534	925	1899	1218	5874	1503	6433	991	76	62	113	73	*92*	56	320	224

[a]Standard deviation
[b]Significant difference ($P \leq .05$) by the t-test

nasal antibody titre to PI-3 virus was higher on day 1 than at day 7 in the S animals.

Prediction of S and W — A discriminant analysis for prediction of S and W on SICK and WELL animals based on parameters X_1 to X_6 yielded a percentage error rate varying from 29% to 40% (Table XI). No trend on error rate was observed in using various subsets of the variables. The day 1 and day 7 data did not provide any real difference in the results. Parameters X_1, X_3 and X_5 are most accurate but the error rate is probably higher than could be used for reliable results.

Dead Animals — The number of observations on those animals which died reduced with time (Table XII). The mean body temperature was not over 105°F and the plasma fibrinogen levels were generally high but the standard deviations were considerable. These values are similar to other SICK

TABLE VI. Individual Lung Scores of 23 SICK and 21 WELL Animals in GROUP I and the Comparisons of Mean Scores

Classification			
SICK		WELL	
Animal Number	Score	Animal Number	Score
4646	30	4977	10
4986	30	4978	7
205	27	4976	4
4983	25	206	4
201	26	4979	3
208	22	143	2
4648	22	141	2
4643	21	4984	2
4993	20	204	2
4642	19	138	2
146	19	144	2
139	19	4980	1
4647	18	4991	1
4982	18	145	1
4987	18	137	1
4992	14	142	1
4981	13	202	1
4989	13	140	0
4994	10	203	0
4995	9	207	0
4990	8	209	0
210	3		
4985	2	mean	5.7
mean	17.6		

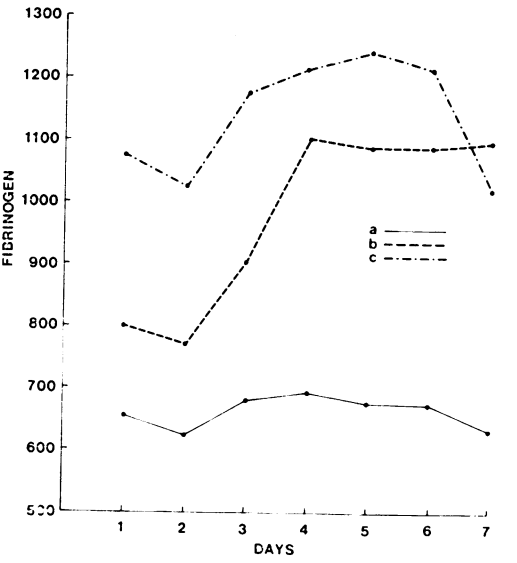

Fig. 4. Daily mean body fibrinogen levels for subgroups a, b and c, divided according to lung scores, from GROUP I (a includes lung scores of 0-10, b, 11-20 and c, 21-30).

animals. The serum antibody titre to *P. hemolytica* on day 1 and day 7 is lower than the mean of the S animals in either GROUPS I or II and the same is true for serum antibody levels to PI-3 virus. The lung scores were available from only three of the 13 animals and these were 30, 30 and 26.

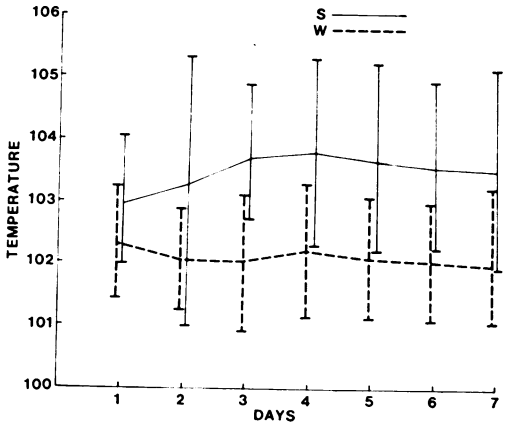

Fig. 5. Daily mean body temperature with standard deviations for SICK and WELL animals in GROUP II.

DISCUSSION

The overall results of this investigation confirm and further support the results of our previous report on factors related to the occurrence of pneumonic pasteurellosis in cattle (26). The present results indicate that some of the parameters measured relate to the degree of pneumonia present in the lungs. In general the animals which developed pneumonia were probably more susceptible to the effects of *P. hemolytica* than those which did not develop pneumonia (Tables III, VIII, X, XII). Based on serum antibody titre to PI-3 virus the response to the virus was generally similar in those animals which developed pneumonia and in those which did not (Tables III, VIII, XII).

The designation of S and W animals is supported by a greater degree of pneumonia in the S than the W animals (Table VI). One of the main objectives of this investigation was to determine whether or not such a relationship existed. The value of the S and W classification in demonstrating differences in animals which develop pneumonia and those which do not is important in establishing the relative significance of factors such as the occurrence of bacterial and viral infection in the two classifications which are probably related to the pathogenesis of the disease. In addition the S and W classification will be very useful in determining the efficacy of experimental control and preventive procedures such as vaccination which might be attempted under field conditions particularly since our results indicate a strong, positive relationship between the S classification and the presence of pneumonia. Until now the evaluation of a preventive measure for "shipping fever" has been assessed only on relatively superficial clinical criteria. Schell *et al* (19) mention the "absence of a reliable assay of protection" in shipping fever.

Increases in body temperature and plasma fibrinogen levels are probably nonspecific indicators of illness in cattle (16) but perhaps fortuitously have been of definite value for our purposes as a means of identifying animals with pneumonia especially the fibrinogen values (Table VI) (Figs. 2, 4, 6).

The high levels of *P. hemolytica* in the nasal flora of S animals compared to W animals indicates this organism proliferates to a much greater extent in the S

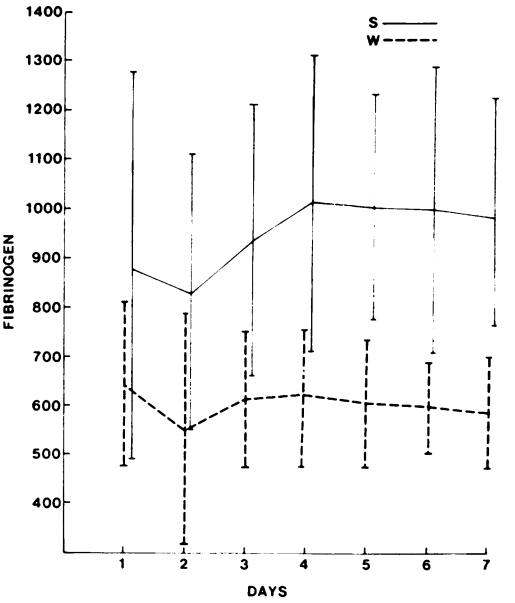

Fig. 6. Daily mean plasma fibrinogen levels with standard deviations for SICK and WELL animals in GROUP II.

TABLE VII. Comparisons of Total and Absolute Differential Leukocyte Counts in Animals of GROUP I Based on Division into 3 Subgroups[a], *a*, *b* and *c* According to Magnitude of Lung Scores

	Total			Band Cells			Segmented Cells			Lymphocytes			Monocytes			Eosinophils		
Day	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*	*a*	*b*	*c*
1	9216	9375	9256	36	53	81	2251	4321	2766	6530	4798	6163	122	31	115	262	139	139
2	8408	8650	9363	22	39	99	1902	2616	2662	6012	5703	6362	122	74	160	316	212	50
3	8652	8610	8950	31	39	137	1862	2505	2349	6326	5852	6320	101	157	57	317	44	87
4	8924	8680	8050	11	38	125	1870	2636	2003	6680	5883	5796	87	73	89	236	37	20
5	9136	8550	8600	12	66	57	1786	2156	2320	6850	6223	6015	129	40	76	318	59	88
6	9108	9840	8429	45	47	191	2217	2717	2551	6418	6945	5599	109	48	35	288	64	53
7	8748	8450	10214	33	39	279	2087	2574	3890	6194	5719	5899	77	43	93	318	66	53

[a] *a* group of 25 animals with lung scores of 0-10
b group of 10 animals with lung scores of 11-20
c group of 9 animals with lung scores of 21-30

TABLE VIII. Mean Colony Counts and Mean Serum Antibody Titres in Subgroups[a] *a*, *b*, *c* of GROUP I Based on Magnitude of Lung Scores

		MCC[b]		Serum Antibody Titres			
Agent	Group	Day 7	SD	Day 1	SD[d]	Day 7	SD
P. hemolytica	*a*	255		63[e]	97	92	108
	b	721		49	48	134	92
	c	560		28	28	157	111
P. multocida	*a*	128					
	b	128					
	c	164					
PI-3 virus	*a*			29	35	35	29
	b			42	78	37	39
	c			25	41	33	47

[a] *a* group has lung scores of 0-10
b group has lung scores of 11-20
c group has lung scores of 21-30
[b] Mean colony count
[c] Reciprocal of dilution
[d] Standard deviation

TABLE IX. Comparisons of Nasal Bacterial Mean Colony Counts Between SICK and WELL Animals of GROUP II

	Classification				Significance		
	SICK		WELL				
Organism	MCC[a]	SD[b]	MCC	SD	DF[c]	t[d]	p[e]
P. hemolytica	366	462	146	321	137	3.28	.00[f]
P. multocida	81	207	138	301	137	1.3	.18[f]

[a] Mean colony count
[b] Standard deviation
[c] Degrees of freedom
[d] t-test value
[e] Actual significance value
[f] Significance value by Welch T Prime test

TABLE X. Comparisons of Mean Antibody Titres Between SICK and WELL Animals of GROUP II on Day 1 and on Day 7

Agent	Day	SICK Mean	SICK SD[a]	WELL Mean	WELL SD	Significance DF[b]	Significance t[c]	Significance p[d]
P. hemolytica — serum	1	30[e]	41	57	80	137	3.25	.00
P. hemolytica — serum	7	118	156	87	121	137	.62	.53[f]
P. hemolytica — nasal	1	0.6	4	0.7	2	137	1.37	.17
P. hemolytica — nasal	7	2	17	0.8	2	137	.69	.49
PI-3 virus — serum	1	18	39	17	25	137	1.36	.18
PI-3 virus — serum	7	21	22	23	27	137	2.11	.04
PI-3 virus — nasal	1	15	25	15	34	137	.12	.91
PI-3 virus — nasal	7	8	11	14	21	137	1.76	.08

[a]Standard deviation
[b]Degrees of freedom
[c]t-test value
[d]Actual significance value
[e]Reciprocal of dilution
[f]Significance value by Welch T Prime test

animals. Theoretically the S animals would have the opportunity to inhale larger numbers of organisms into their lungs than the W animals (11). The increase in serum antibody to *P. hemolytica* in the S animals between days 1 and 7 also indicates a stronger challenge from this organism than occurred in the W animals. The lower serum antibody levels to *P. hemolytica* in the S animals on day 1 (Tables III, VIII, XII) could indicate a decreased period of time exposure to the organism compared to W animals prior to arrival. The S animals would therefore be more susceptible to the effects of *P. hemolytica* assuming that the serum IHA antibody titres to *P. hemolytica* are an indication of resistance.

However a relationship between IHA antibody titre to *P. hemolytica* and resistance has not been specifically verified in cattle (2, 3) although some reports contain information related to this point. Baldwin *et al* (1) were able to cause clinical illness with *P. hemolytica* via intratracheal inoculation in calves with levels of serum IHA antibody less than 1:10. Duncan and Thomson (8) also caused clinical signs of pneumonia with aerosols of live *P. hemolytica* in calves with no serum IHA antibody titres to *P. hemolytica*. The IHA test is an indication of exposure to capsular antigens. Experiments to relate these titres to immune status in mice have not been conclusive: "The results very clearly point to the existence of factors other than capsular and somatic antigens demonstrable by simple laboratory typing that determine specificity of immunity" (15), a statement which is consistent with the findings of Cameron (3). Nevertheless there has not been a report of a better method of indicating exposure to live *P. hemolytica* organism and probable protection in cattle (8, 10).

The general results of our investigation would support the concept that these antibody titres are an indication of resistance in that animals with low titres are the ones which tend to develop pneumonia compared to those with higher titres (Tables III, VIII, XII). We are unable to evaluate the significance of nasal antibody to *P. hemolytica*. The levels of nasal antibody to *P. hemolytica* induced experimentally (8) have not been found in our experimental field animals and no explanation is apparent.

TABLE XI. Percentage Error in Selecting SICK and WELL Animals from GROUP II Determined by Discriminant Analysis

Parameter	Percent Error Day 1 Data	Percent Error Day 7 Data
X_1, X_3	35	32
X_1, X_3, X_5	36	29
X_1, X_3, X_5, X_6	32	35
X_1, X_3, X_4, X_5, X_6	35	35
X_1, X_2, X_3, X_4, X_5, X_6	38	41

X_1 — MCC *P. hemolytica*
X_2 — MCC *P. multocida*
X_3 — Serum antibody *P. hemolytica*
X_4 — Nasal antibody *P. hemolytica*
X_5 — Serum antibody PI-3 virus
X_6 — Nasal antibody PI-3 virus

It is of interest that it was just twenty years ago that a large scale study of the value of *P. multocida* bacterin and antiserum was carried out on a group of 9,000 animals (18). That study and Carter's work (4, 5) seem to have been responsible for focusing attention toward *P. hemolytica* (7).

The W animals had a significantly higher serum antibody level to PI-3 virus on day 7 than the S animals in GROUP II (Table X) but not in GROUP I (Table IV). However the W animals in GROUP II had a significantly higher nasal antibody level to PI-3 virus on day 7 than day 1 (Table X). It has been demonstrated that nasal antibody to PI-3 virus is a better indicator of protection against PI-3 infection in cattle than is serum antibody (9). Our serological results do not indicate an active PI-3 virus infection or that there was a relationship between the degree of pneumonia and PI-3 virus infection. However Sweat (22, 23) demonstrated clear serological evidence of active PI-3 virus infection in recently shipped calves some of which had clinical signs of shipping fever and at least one died with fibrinous pneumonia. He considered an HI antibody titre of 1:20 to be significant as an indication of previous PI-3 virus infection. He also reported that HI antibody levels in calves after nursing were about 1:50 and then declined to about zero at six to eight months of age. A rise in antibody levels to about 1:30 to 1:40 usually occurred again when the cattle were weaned. About 86 to 100% of a group of 200 calves developed titres between 1:20 and 1:80 during an outbreak of shipping fever in which the virus was isolated from nasal swabs of several calves. Vaccination can result in serum HI titres of up to 1:200 (9) but natural infection may only give rises of 1:40 to 1:80. The mean HI titres in our animals were below 1:40. At their age passively secured antibody should not have been a factor. Compared to Sweat's animals (22, 23) ours did not have a significant PI-3 infection and animals of our groups were dying of severe fibrinous pneumonia.

However the serum antibody titres to PI-3 virus in our animals do not rule out an effect of PI-3 virus on the development of pneumonia. It has been demonstrated (13, 27) that a virus infection in the lung of experimental animals can markedly impair the clearance of inhaled bacteria from the lung particularly one week after the virus infection. Therefore if PI-3 virus infection had occurred about one or two weeks prior to shipment or arrival then it could possibly affect clearance and become highly significant in the pathogenesis of the pneumonia. However the antibody levels to PI-3 virus (Tables III, V, X, XII) do not indicate a recent or active infection at least when compared to Sweat's results (23). An effect of PI-3 virus on the pulmonary clearance of *P. hemolytica* in cattle has not been demonstrated (10) but is still under investigation in our laboratories. If PI-3 virus is demonstrated to have an effect on pulmonary clearance of *P. hemolytica* in

TABLE XII. Observations on Animals Which Died or Were Killed *in extremis* in GROUPS I and II

Day	Number of Observations	Temperature Mean	Temperature SD[a]	Fibrinogen Mean	Fibrinogen SD	Mean Colony Count *P. hem.*[b]	Mean Colony Count *P. mult.*[c]	Antibody Titres *P. hem.* S[d]	*P. hem.* N[e]	PI-3 S	PI-3 N
1	13	103.8	1.9	929	311			14[f]	.2	4	26
2	12	103.3	2.8	938	338						
3	10	104.6	1.5	1052	350						
4	9	104.7	1.8	1166	283						
5	6	104.6	1.1	1343	261						
6	6	103.5	1.9	1169	301						
7	3	103.9	2.6	1175	494	637	59	61	.4	5	11

[a]Standard deviation
[b]*P. hemolytica*
[c]*P. multocida*
[d]Serum
[e]Nasal
[f]Reciprocal of titres

cattle then the effect could probably be blocked by aerosol immunization with PI-3 virus. However if the virus is demonstrated not to affect clearance, then clearly, preventive measures must be aimed at *P. hemolytica* alone because aerosol immunization with this agent could probably protect the respiratory tract from subsequent challenge by the homologous organism (10, 13). Our results support other reports by Carter (4, 5) and Collier (6, 7) and the description of Jubb and Kennedy (14) concerning "shipping fever" which indicate that *P. hemolytica* is the agent which causes severe clinical illness and death even though other factors may be responsible for its rapid growth in susceptible animals. However a detailed microbiological examination of lungs in the early stages of pneumonia has not to affect clearance, then clearly, preyet undetermined factors may influence the early stages of the disease.

The comparisons of the mean absolute leukocyte counts indicate little difference between S and W animals except low eosinophil counts in the S animals which probably means higher levels of circulating adrenal steroids and therefore greater stress in the S animals although we have not confirmed this. Carter and McSherry also noted an eosinopenia in field cases (5).

The designation of lung scores has allowed direct comparison between the degree of pneumonia and the parameters which might be significant in the pathogenesis as mentioned previously. The arbitrary division into subgroups *a*, *b* and *c* allowed a closer examination of this relationship although in general the animals in subgroup *a* were very similar to the W animals and those in *b* and *c* similar to the S animals. The animals which died (Table XII) had values for the antibody titres and MCC generally similar to the subgroup *c* animals which had the greatest degree of pneumonia (Table VIII).

Considerable time and effort were expended to relate and rank the significance of parameters X_1 to X_6 to the classification of S and W without the use of temperature and fibrinogen values and to reduce the number of tests required for the establishment of the S and W criteria. The purpose was to more closely identify the most significant microbiological parameters and use only those for larger scale field trials in evaluation of the effects of preventive or control procedures. The results indicate that probably the measurement of temperature, fibrinogen, MCC of *P. hemolytica* and serum antibody levels to *P. hemolytica* and PI-3 virus would be required in such an evaluation over a one week period as carried out for GROUP I animals.

It should be pointed out that our results relate to feedlot cattle and their circumstances of weaning, travel and a new environment and not to enzootic pneumonia in dairy calves (28, 30) which is a different clinical and pathological disease.

ACKNOWLEDGMENTS

The work was supported by the Ontario Ministry of Agriculture and Food and by Agriculture Canada. The assistance of Susanne Foster, who completed most of the data, Brendan McCann, Florence Rilett, Bill Turner and Bob McCall is appreciated. S. S. Swaminathan, N. Ison and John Tofflemire of the Institute of Computer Science made the pooling of data and much of the analysis possible.

REFERENCES

1. **BALDWIN, D. E., B. S. MARSHALL and G. E. WESSMAN.** Experimental infection of calves with myxovirus parainfluenza-3 and **Pasteurella hemolytica.** Am. J. vet. Res. **28**: 1773-1782. 1967.
2. **BIBERSTEIN, E. L., M. GILLS and H. KNIGHT.** Serological types of **Pasteurella hemolytica.** Cornell Vet. **50**: 280-300. 1960.
3. **CAMERON, C. M.** The haemagglutination test and immunity to **Pasteurella hemolytica.** J.S. Afr. vet. med. Ass. **37**: 165-170. 1960.
4. **CARTER, G. R.** Observations on the pathology and bacteriology of shipping fever in Canada. Can. J. comp. Med. **18**: 359-364. 1954.
5. **CARTER, G. R. and B. J. McSHERRY.** Further observations on shipping fever in Canada. Can. J. comp. Med. **19**: 177-181. 1955.
6. **COLLIER, J. R., W. W. BROWN and T. L. CHOW.** Microbiological investigation of shipping fever of cattle. J. Am. vet. med. Ass. **140**: 807-810. 1962.
7. **COLLIER, J. R.** Pasteurellae in bovine respiratory disease. J. Am. vet. med. Ass. **152**: 824-828. 1968.
8. **DUNCAN, J. R. and R. G. THOMSON.** Influence of immunization procedures on upper respiratory tract immunity in cattle Can. J. comp. Med. **34**: 94-100. 1970.
9. **FRANK, G. H. and R. G. MARSHALL.** Relationship of serum and nasal secretion — neutralizing antibodies in protection of calves against Parainfluenza-3 virus. Am. J. vet. Res. **32**: 1707-1713. 1971.
10. **GILKA, F. and R. G. THOMSON.** The effect of edema, hydrocortisone acetate, concurrent viral infection and immunization on the clearance of **Pasteurella hemolytica** from the bovine lung. Can. J. comp. Med. **38**: 251-259. 1974.
11. **GREY, C. L. and R. G. THOMSON.** **Pasteurella hemolytica** in the tracheal air of calves. Can. J. comp. Med. **35**: 121-128. 1971.
12. **HENRY, R. J.** Clinical Chemistry, Principles and Techniques. 2nd Ed. p. 228. New York: Harper & Row. 1964.

13. **JAKAB, G. J. and G. M. GREEN.** Immune enhancement of pulmonary bactericidal activity in murine virus pneumonia. J. clin. Invest. **52**: 2878-2884. 1973.

14. **JUBB, K. V. F. and P. C. KENNEDY.** Pathology of Domestic Animals. Vol. 1. 2nd Ed. New York: Academic Press. 1971.

15. **KNIGHT, H. D., E. L. BIBERSTEIN and M. ALLISON.** The role of capsular and somatic antigens in immunization of mice against **Pasteurella hemolytica** infection. Cornell Vet. **59**: 55-64. 1969.

16. **McSHERRY, B. J., F. D. HORNEY and J. J. DE GROOT.** Plasma fibrinogen levels in normal and sick cows. Can. J. comp. Med. **34**: 191-197. 1970.

17. **MORSE, E. E., S. PANEK and R. MENGA.** Automated fibrinogen determination. Am. J. clin. Path. **55**: 671-676. 1971.

18. **RICE, C. E., M. BEAUREGARD and T. K. MAYBEE.** Survey of shipping fever in Canada: Serological studies. Can. J. comp. Med. **19**: 329-349. 1955.

19. **SCHELL, K., R. P. SANDERSON, J. W. WHALEN and J. L. BITTLE.** The antigenicity of multivariant vaccines for bovine respiratory disease. Cornell Vet. **62**: 101-109. 1972.

20. **SEVER, J. L.** Application of a microtechnique to viral serological investigations. J. Immun. **88**: 320-329. 1962.

21. **STEEL, R. G. D. and J. H. TORRIE.** Principles and Procedures of Statistics. New York: McGraw Hill. 1960.

22. **SWEAT, R. L.** Isolation of myxovirus parainfluenza-3 from bovine respiratory diseases. J. Am. vet. med. Ass. **150**: 172-177. 1967.

23. **SWEAT, R. L.** Epizootiologic studies of bovine myxovirus parainfluenza-3. J. Am. vet. med. Ass. **150**: 178-183. 1967.

24. **THOMSON, G.** Aspects of the bovine coagulation system in experimental endotoxemia. Thesis, University of Guelph, 1973.

25. **THOMSON, G., B. J. McSHERRY and V. E. O. VALLI.** Evaluation of a rapid method of determination of plasma fibrinogen. Can. J. comp. Med. **38**: 266-270. 1974.

26. **THOMSON, R. G., M. L. BENSON and M. SAVAN.** Pneumonic pasteurellosis of cattle: Microbiology and immunology. Can. J. comp. Med. **33**: 194-206. 1969.

27. **THOMSON, R. G. and F. GILKA.** A brief review of pulmonary clearance of bacterial aerosols emphasizing aspects of particular relevance to veterinary medicine. Can. vet. J. **15**: 99-107. 1974.

28. **THOMSON, R. G.** Pathology and pathogenesis of the common diseases of the respiratory tract of cattle. Can. vet. J. **15**: 249-251. 1974.

29. **WELCH, B. L.** Significance of the differences between two means when the population variances are unequal. Biometrika **29**: 350-362. 1937.

30. **WRAY, C. and D. A. THOMPSON.** An epidemiological study of **Pasteurella haemolytica** in calves. Br. vet. J. **129**: 116-123. 1973.